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TITLE: Cancer-Associated Macrophagelike (CAML) Cells to Enhance Detection of Early-Stage Lung Cancer and Relapse After Definitive Treatment

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14. ABSTRACT Lung cancer is the number one cause of cancer death in the United States. Low dose CT (LDCT) was recommended to screen for lung cancer for smokers by the US Preventative Services Task Force. However, there are several problems with the current screening paradigm. Most critically, over 39% of screened subjects were determined to have positive screens with 96.4% false positive. This very high false positive rate results in several critical problems including the requirement for further testing (scans, biopsies), the potential of loss to follow-up, the possibility of false negative biopsy and the resultant patient stress and anxiety. While lung nodules <0.8 cm are considered low-risk findings and nodules >3.0 cm high-risk, nodules between from 0.8-3.0 cm have been described as "indeterminate" and represent a management challenge. Therefore, there is a substantial need for a method to enrich the population of patients identified as likely to have malignancy and exclude those who have nodules not likely to have malignancy.						
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Title: Cancer Associated Macrophage-Like (CAML) Cells to Enhance Detection of Early Stage Lung Cancer

Company: Creatv MicroTech (Creatv)

1. INTRODUCTION:

Lung cancer is the number one cause of cancer death in the United States. Low dose CT (LDCT) was recommended to screen for lung cancer for smokers by the US Preventative Services Task Force. However, there are several problems with the current screening paradigm. Most critically, over 39% of screened subjects were determined to have positive screens with 96.4% false positive. This very high false positive rate results in several critical problems including the requirement for further testing (scans, biopsies), the potential of loss to follow-up, the possibility of false negative biopsy and the resultant patient stress and anxiety. While lung nodules <0.8 cm are considered low-risk findings and nodules >3.0 cm high-risk, nodules between from 0.8-3.0 cm have been described as “indeterminate” and represent a management challenge. Therefore, there is a substantial need for a method to enrich the population of patients identified as likely to have malignancy and exclude those who have nodules not likely to have malignancy.

Creatv identified a very large cell in the blood of cancer patients. These cells had not been previously analyzed in published in literature. We gave them the name CAMLs (cancer associated macrophage-like cells). They are specialized myeloid polynucleated cells that emanate from primary tumor masses and transit the circulation of cancer (Fig. 1) in a variety of malignancies. CAMLs are absent in healthy controls and are rare in persons with benign masses. CAMLs are polynucleated and very large, 25-300 microns in size. They are isolated from whole peripheral blood using Creatv’s CellSieve™ platform, a filter based system with 7 micron pore size and uniform distribution of the pores over a 9 mm area.

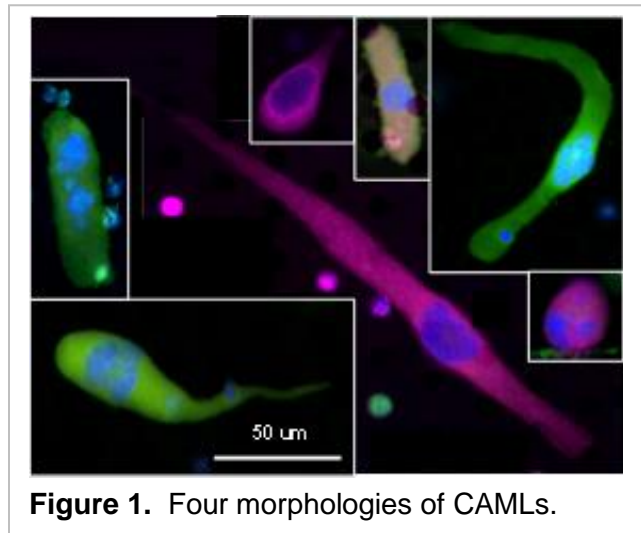


Figure 1. Four morphologies of CAMLs.

The research goal is to determine if CAMLs can be used to determine which patients that present with nodules actually have lung cancer. Detecting CAMLs in blood is a liquid biopsy method to enrich the population of patients identified as likely to have malignancy and exclude those who have nodules not likely to have malignancy.

2. KEYWORDS:

Pulmonary nodules, low dose CT, LDCT, lung cancer, cancer associated macrophage-like cells, CAMLs, liquid biopsy

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

The **Specific Aims** of this proposal are:

1. Determine the prevalence of CAMLS in patients with pulmonary nodules.
2. Determine the positive and negative predictive value of CAMLS in patients with pulmonary nodules who undergo biopsy.
3. Model combinations of clinical factors with the presence/absence of CAMLS to refine strategies for assessment of patients with pulmonary nodules.

- **What was accomplished under these goals?**

To accomplish these aims, a total of 1000 patients will be enrolled. Two tubes of blood will be collected from patients whose LDCT detected nodules. If the patient is biopsied or other diagnostic/therapeutic procedure is performed, two tubes of blood will be collected again before this procedure.

The blood will be filtered to collect CAMLS using the CellSieve™ microfiltration platform followed by staining for markers to identify CAMLS. For each patient, one tube of blood will be processed at FCCC and one at Creatv's New Jersey lab. Both slides will be read by Creatv.

The markers CK 8, 18, 19 are widely accepted as marker for epithelial cells, such as cells from lung cancer. However, CK 8, 18, 19 are typically weak for lung cancer, so another marker is used to identify CAMLS. After analyzing >50 markers and >1000 CAMLS, CD31 marker is found to be high expressing in >98% of CAMLS.

An aspect of the assay is to determine that the CAMLS are from lung cancer, not some other cancer. To achieve this goal, we decided to use the marker thyroid transcription factor 1 (TTF1) to identify lung cancer. Table 1 shows that TTF1 provides high specificity for lung cancer.

Site	No	% Positivity of each marker									
		PSA	TTF1	QCDFP-15	CDX2	CK20	CK7	ER	Mamot helin	CA 125	Lysozyme
Breast	52	0	0	49	0	0	87	79	4	13	11
Colon	69	0	0	6	86	78	3	1	7	1	43
Lung	72	0	90	3	1	1	90	6	36	38	35
Ovary serous	27	0	0	4	0	0	93	81	96	93	7
Ovary mucinous	14	2	0	14	14	21	57	50	36	36	29
Pancreas	80	0	4	1	2	15	94	0	49	50	51
Stomach	52	2	2	0	21	21	50	0	19	8	81
Prostate	25	100	8	4	0	0	0	8	0	0	4

Table 1. Relation of tissue markers to cancer. TTF1 provides very high specificity. (Ref.: Dennis, et al., Clin Cancer Res 2005.)

We proceeded to develop a 5 marker assay to identify CAMLs: DAPI, CK 8, 18, 19 (FITC), CD31 (PE), CD45 (cyanine5), TTF1 (Cy7).

This assay requires 5 fluorescent channels. None of our microscopes had fluorescent filter cubes for Cy7 channel. We determined that the appropriate fluorescent cube is: Chroma IN009878 part number 49007

Excitation wavelength: center 710 nm, 75 nm wide.

Emission wavelength: center 810 nm, 90 nm wide.

This filter cube has minimal cross over with nearby Cy5 fluorescence.

To develop the assay, the following cells were selected for the assay development:

- Lung cancer cell line H441 (TTF1 high)
- Breast cancer cell line MCF7 (TTF1 low) (CK 8, 18, 19 high)
- Human umbilical vein endothelial cells (HUVEC) (CD31 high)
- PBMC (CD45 high)

We have MCF7 cell line and PBMCs. The H441 cell line and HUVEC were ordered. Cell lines H441 and MCF7 were cultured, collected and fixed.

To develop the TTF1 assay, rabbit TTF1 antibody (clone D2E8) and secondary antibodies with different fluorescent dyes were evaluated. Initial assay was to determine if TTF1 with secondary antibodies can be seen on the microscope.

- The Leica microscope in our MD lab, however, can only see extremely weak signals in the Cy7 channel. Leica told us the reason and there is no way to change that. In Maryland lab, we grow cell lines, test reagents and make kit production.
- Olympus microscope in our NJ lab can see TTF1 secondary with Cy7 or AF750. This is important, because patient slides are read in the NJ lab.

AF750 was chosen to be the fluorescent dye, because Cy7 dye has higher background.

Once an optimal assay condition was determined, the specific CellSieve™ kits for lung cancer was produced, which included an antibody cocktail (cytokeratins (CK) 8, 18, 19, CD31, CD45, TTF1) a blocking reagent, a secondary antibody and other standard buffers and filters, were manufactured at Creatv's Rockville lab.

In order not to miss any patient samples, two test kits using secondary for TTF1 (10 tests/kit) were shipped to Kathy Alpaugh at the Fox Chase Cancer Center (FCCC) Protocol Support Laboratory, and two kits were shipped to Creatv's New Jersey Lab. The technician at FCCC was trained to process the patient samples.

After this, we developed the assay for TTF1 using primary conjugated antibodies, in order to simplify the assay and reduce the running time. Custom conjugation of the TTF1 antibody was ordered. AF750 directly conjugated to TTF1 was obtained. Future kits will use primary antibodies.

Patient Samples:

Philadelphia VA did not get IRB approval in Year 1. FCCC received their IRB approval late in Year 1. Only 4 patient samples (8 slides) from FCCC were received in Year 1.

- **What opportunities for training and professional development has the project provided?**

Nothing to Report.

- **How were the results disseminated to communities of interest?**

Nothing to Report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

Philadelphia VA's IRB is now approved. The samples to be processed will be ramping up.

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to Report.

- **What was the impact on other disciplines?**

Nothing to Report.

- **What was the impact on technology transfer?**

Nothing to Report.

- **What was the impact on society beyond science and technology?**

Nothing to Report.

5. CHANGES/PROBLEMS:

None.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Nothing to Report.

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	<i>Cha-Mei Tang</i>
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	<i>none</i>

Nearest person month worked:	0.5
Contribution to Project:	<i>Performed assay development and patient samples</i>
Funding Support:	W81XWH1810197

Name:	<i>Daniel Adams</i>
Project Role:	<i>Director of Clinical R&D</i>
Researcher Identifier (e.g. ORCID ID):	<i>none</i>
Nearest person month worked:	1
Contribution to Project:	<i>Performed assay development and processed patient samples</i>
Funding Support:	W81XWH1810197

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report.

- **What other organizations were involved as partners?**

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**

Not Applicable

- **QUAD CHARTS:**

Not Applicable

9. APPENDICES:

None